ESS Method 230.1: Total Phosphorus and Total Kjeldahl Nitrogen, Semi-Automated Method

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1.0 Scope and Application

1.1 This method covers the determination of total Kjeldahl nitrogen and total phosphorus in drinking, surface and waste waters. The operating range is 0.1 to 10.0 mg N/L and 0.02 to 2.00 mg P/L.

1.2 The digestion converts nitrogen compounds such as amino acids, proteins and peptides to ammonia, but may not convert all amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines.

2.0 Summary of Method

2.1 Organic nitrogen and phosphorus compounds are digested with a sulfuric acid solution containing potassium sulfate and using mercuric sulfate as a catalyst:

$$H_2SO_4 + \textit{organic nitrogen} \xrightarrow[K_2SO_4]{Hg} (NH_4)_2SO_4$$

$$H_2SO_4 + \textit{organic phosphorus} \xrightarrow[K_2SO_4]{Hg} K_3PO_4$$

2.2 The digested solution is analyzed spectrophotometrically as ammonia and phosphate using an automated system with an internal neutralization step.

2.2.1 In the TKN determination, the NH_3 is treated with sodium hypochlorite and sodium phenolate to form indophenol blue. Sodium nitroprusside is used to intensify the color. The intensity of the color is directly related to the concentration of TKN.

2.2.2 In the TP determination, the PO_4 reacts with ammonium molybdate in the presence of H_2SO_4 to form a phosphomolybdenum complex. Potassium antimonyl tartrate and ascorbic acid are used to reduce the complex, forming a blue color which is proportional to the TP concentration.

3.0 Sample Handling and Preservation

3.1 The samples are collected in 250 mL high density polyethylene containers.

3.2 Samples are preserved in the field with 2 mL of 12.5% H_2SO_4/250 mL (1 mL of conc. H_2SO_4/L, pH <2) and stored at 4°C.

3.3 Samples such as sewage, paper mill wastes, etc., which contain settleable materials must be homogenized before withdrawing an aliquot for analysis.

4.0 Interferences

4.1 A sodium citrate - sodium potassium tartrate complexing reagent is used in the TKN manifold to minimize the interference caused by the precipitation of metal ions.

4.2 A sodium chloride reagent is used to prevent the reduction of mercuric ions in the TP manifold.

5.0 Apparatus

5.1 Technicon BD-40 Block Digestor (two units)

5.2 Technicon #114-0024-02 glass tubes

5.3 Technicon rack #114-0009-02

5.4 Teflon boiling chips

5.5 Sonicator Cell Disruptor (Heat Systems Ultrasonics, Inc., Plainview, NY)

5.6 Oxford 500 and 1000 µL pipet with disposable polypropylene tips

5.7 Vortex-genie mixer

5.8 Labindustries 10 mL capacity Repipet (2 units)

5.9 Culture tubes, 15 x 85 mm disposable glass

5.10 Technicon AutoAnalyzer II system consisting of:

5.10.1 Sampler IV with a 30/h (2:1) Cam

5.10.2 Proportioning Pump III with dilution manifold

5.10.3 Modified ammonia manifold

5.10.4 Modified orthophosphate manifold

5.10.5 Colorimeter equipped with 15 mm flowcells and 630 nm interference filters for TKN

5.10.6 Colorimeter equipped with 50 mm flowcells and 880 nm interference filters for TP

5.10.7 Printer/Plotter

6.0 Reagents

6.1 Digestion acid solution

6.1.1 Sulfuric acid, 6 N: Dilute 167 mL of concentrated H_2SO_4 to 1 L with Milli-Q water (Milli-Q reagent grade water system, Millipore Corp.).

6.1.2 Mercuric oxide solution: Dissolve 2.0 g of HgO in 25 mL of 6 N H_2SO_4 (Section 6.1.1).

6.1.3 Potassium sulfate solution: Partially dissolve 134 g of K_2SO_4 in 500 mL of Milli-Q water.

6.1.4 Add 200 mL of concentrated H_2SO_4 to the K_2SO_4 solution (Section 6.1.3) and stir until K_2SO_4 is dissolved.

6.1.5 Add HgO solution (Section 6.1.2) to the K_2SO_4 acid solution (Section 6.1.4), cool slightly, dilute to 1 L and store above 20°C.

6.2 Digestion tube dilution water: Use Milli-Q water that is N and P free.

6.3 Sampler wash solution: Add 70 mL of conc. H_2SO_4 to 1500 mL of Milli-Q water and dilute to 2 L.

6.4 Dilution loop solution: Add 50 mL of 20% w/v NaOH to 1500 mL of Milli-Q water and dilute to 2 L.

6.5 TKN Reagents

6.5.1 Complexing reagent: Dissolve 33 g of sodium potassium tartrate ($NaKC_4H_4O_6 \bullet 4H_2O$) and 24 g of sodium citrate ($Na_3C_6H_5O_7 \bullet 2H_2O$) in 900 mL of Milli-Q water and dilute to 1 L. Add 0.25 mL of Brij-35.

6.5.2 Alkaline phenol: Using a 1 L Erlenmeyer flask, dissolve 83 g of phenol in about 50 mL Milli-Q water. Cautiously add with mixing 180 mL of 20% w/v NaOH. Cool, dilute to 1 L, and filter through a glass fiber filter. 2 L can be made at one time.

6.5.3 Sodium hypochlorite solution: Dilute 200 mL of commercial bleach containing 5.25% available chlorine (e.g. Clorox) to 1 L with Milli-Q water and filter through a 0.45 µm membrane filter. Store at 4°C.

6.5.4 Sodium nitroprusside: Dissolve 0.5 g of ($Na_2Fe(CN)_5NO \bullet 2H_2O$) in 900 mL of Milli-Q water and dilute to 1 L. Reagent is light sensitive, store in dark containers.

6.6 Phosphorus Reagents

6.6.1 Diluent water solution: Dissolve 5 g of sodium chloride in 900 mL of Milli-Q water and dilute to 1 L. Add 0.25 mL of Levor IV.

6.6.2 Stock Solution A, 4.9 N Sulfuric acid: Add 136 mL of conc. H_2SO_4 to 800 mL of Milli-Q

water. Cool and dilute to 1 L.

6.6.3 Stock Solution B, Ammonium molybdate: Dissolve 40 g of $(NH_4)_6Mo_7O_{24}•4H_2O$ in 900 mL of Milli-Q water and dilute to 1 L. Store at 4°C.

6.6.4 Stock Solution C, Ascorbic acid: Dissolve 9 g of ascorbic acid ($C_6H_8O_6$) in 400 mL of Milli-Q water and dilute to 500 mL. Store at 4°C. Keep well stoppered. Prepare fresh monthly or as needed.

6.6.5 Stock Solution D, Antimony potassium tartrate: Dissolve 3.0 g of $K(SbO)C_4H_4O_6•1/2H_2O$ in 800 mL of Milli-Q water and dilute to 1 L. Store at 4°C.

6.6.6 Combined color reagent: Combine the following solutions in order, mixing after each addition: 50 mL of Stock A, 15 mL of Stock B, 30 mL of Stock C and 5 mL of Stock D. Prepare fresh daily.

6.7 Standard Solutions

6.7.1 Stock nitrogen standard: Dissolve 1.050 g of glutamic acid (dried at 105°C for 1 h) in 900 mL of Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L. 1.0 mL = 0.100 mg N (100 mg N/L).

6.7.2 Stock phosphorus standard: Dissolve 0.4394 g of potassium phosphate monobasic (KH_2PO_4) (dried at 105°C for 1 h) in 900 mL of Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L. 1.0 mL = 0.100 mg P (100 mg P/L).

6.7.3 Stock nitrogen spike solution (Nicotinic acid, NA): Dissolve 0.8790 g of NA (dried at 105°C for 1 h) in 900 mL of Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L. 1.0 mL = 0.1 mg N (100 mg N/L).

6.7.3.1 Working NA solution (5.0 mg N/L): Add 50 mL stock NA solution (Section 6.7.3) to 900 mL Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L.

6.7.4 Stock phosphorus spike solution (Adenosine 5'-monophosphate, AMP): Dissolve 0.2242 g AMP (dried at 105°C for 1 h) in 900 mL of Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L. 1.0 mL = 0.02 mg P (20 mg P/L).

6.7.4.1 Working AMP solution (1.0 mg P/L): Add 50. mL stock AMP solution (Section 6.7.4) to 900 mL Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L.

6.7.5 Working standards: Prepare the following standards by adding appropriate amounts of the stock standards to 500 mL of Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to L:

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Conc.		mL Standard Soln./L	
mg P/L	mg N/L	P stock	N stock
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0.40	2.00	4.00	20.00
1.00	5.00	10.00	50.00
2.00	10.00	20.00	100.00

7.0 Procedure

7.1 All glassware must be rinsed with 1:1 HCl to prevent phosphorus contamination. No commercial detergents may be used.

7.2 Put four to eight Teflon boiling chips in each tube.

7.3 Homogenize any non-uniform samples, such as sewage, paper mill wastes, farm wastes, etc. with the Sonicator Cell Disruptor for about 30 seconds.

7.4 Transfer sample to the digestion tube using a large orifice pipet. Determine the sample volume from the NH_3-N and Diss-P concentrations and the following guide. Do not use more than 20 mL.

Sample Volume (mL)	TKN Range (mg N/L)	TP Range (mg P/L)
20	0.05 - 5	0.01 - 1
10	0.1 - 10	0.02 - 2
5	0.2 - 20	0.04 - 4
2	0.5 - 50	0.1 - 10
1	1.0 -100	0.2 - 20

7.5 Quick Phosphorus Test: When the concentration of dissolved phosphorus is unknown, a quick test should be performed to determine the volume needed for the total phosphorus analysis.

7.5.1 Reagents: (A) Ammonium molybdate reagent: Dissolve 25 g $(NH_4)_6Mo_7O_{24}\bullet 4H_2O$ in 175 mL Milli-Q water. *Cautiously* add 280 mL concentrated H_2SO_4 to 400 mL Milli-Q water. Cool, add the molybdate solution, and dilute to 1 L. (B) Stannous chloride reagent: Dissolve 2.5 g of fresh $SnCl_2\bullet H_2O$ in 100 mL glycerol. Heat in a water bath and stir with a glass rod until dissolved.

7.5.2 Procedure: Dilute a test volume to 25 mL with Milli-Q water. Add 1 mL of ammonium molybdate reagent (A) and mix well with vortex mixer. Add 1 drop of the stannous chloride reagent (B) and mix well. A pale blue color denotes that the test volume is a suitable approximate volume of sample. A medium to dark blue color means a smaller volume should be used. Another quick test, using a smaller volume, can be performed if uncertain about the dilution needed.

7.6 Each set of 40 samples should include the following: two blanks (10 mL Milli-Q water), five intercalibration standards, one spiked sample, and three duplicate samples.

7.6.1 A typical run pattern is as follows:

1. Standard, TKN = 10.0, TP = 2.0 as Primer
2. Standard, TKN = 10.0, TP = 2.0
3. Standard, TKN = 7.5, TP = 1.5
4. Standard, TKN = 5.0, TP = 1.0
5. Standard, TKN = 5.0, TP = 1.0
6. Standard, TKN = 2.0, TP = 0.5
7. Blank
8. Blank

9-40. Samples, spiked sample, duplicate samples, NA and AMP, randomly distributed.

7.7 Add 2 mL of digestion acid solution (Section 6.1) to each tube using the repipet and mix thoroughly with vortex mixer.

7.8 Transfer the tubes to the rack provided with the Block Digestor.

7.9 Place the rack of 40 tubes in the first Block Digestor, preheated to 200°C, for about 60 minutes or until all water has evaporated.

7.10 When evaporation is complete, transfer the rack of tubes to the second Block Digestor, preheated to 380°C, and time the digestion for 75 minutes. Remove the tubes from the digestor and allow to cool for 10-15 minutes.

7.11 Using a second Repipet dispenser, add 10.0 mL of Milli-Q water to each tube and mix well using the vortex mixer. Transfer the solution to a clean 15 x 85 mm test tube, and cover with Parafilm (American Can Co., Greenwich, CT). If the sample contains clay-like particulates, allow to settle overnight or centrifuge until clear. If the sample contains dark material, the sample must be redigested at a greater dilution.

7.12 Place the tubes in the Sampler IV tray.

7.13 Set up the manifolds. Allow the colorimeters, and printer to warm up for one-half hour.

7.14 Load Sampler according to the CFDA Tray Protocol.

7.15 Analyze according to procedures described in the LIMS-CFDA Methods manual and General AutoAnalyzer Procedures.

8.0 Calculation

8.1 The total phosphorus and total Kjeldahl nitrogen concentrations are obtained directly from the LIMS plotter.

8.2 If a sample is outside the optimal operating range, select an appropriate sample volume (Section 7.4) and repeat the analysis.

9.0 Precision and Accuracy

Precision and accuracy data are available in the Inorganic Chemistry Unit Quality Assurance Manual.

10.0 References

10.1 Jirka, A.M., Carter, M.J., May, D., and Fuller, F.D., "Ultramicro Semiautomated Method for Simultaneous Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Wastewaters", Environ. Science and Technology. 10:1038-1044, (1976).

10.2 Bowman, G.T and Delfino, J.D., "Determination of Total Kjeldahl Nitrogen and Total Phosphorus in Surface Water and Wastewater", JWPCF. 54,1324 (1982).